Pulmonary arterial hypertension (PH) is primarily a disease of the small pulmonary arteries, characterized by vascular proliferation, remodeling and progressive increases in pulmonary vascular resistance, ultimately leading to right ventricular failure and death. The increases in pulmonary vascular resistance are attributed to endothelial dysfunction resulting in vasoconstriction, remodeling of the pulmonary vessel wall and thrombosis. The vascular endothelium synthesizes and releases a wide range of vasodilators and vasoconstrictors that play key role in the local regulation of vascular tone including endothelin-1 (ET-1), a highly potent peptidic vasoconstrictor and smooth muscle mitogen. Moreover, in patients with PH, an elevated level of ET-1 has been found in the lung and has been shown to be inversely proportional to the magnitude of pulmonary blood flow and cardiac output. Shear stress, vasoconstrictors, growth factors, and cytokines stimulate ET-1 synthesis, whereas nitric oxide, prostacyclin, and atrial natriuretic peptide inhibit its synthesis. ET-1 is predominantly synthesized in endothelial cells of blood vessels and in smooth muscle and exerts its vascular effects through activation of ET_A (located on smooth muscle cells) and ET_B receptors (located on vascular endothelial cells and smooth muscle cells). Binding of ET to the ET_A receptor leads to the activation of phospholipase C causing hydrolysis of phosphatidylinositol and generation of cytosolic inositol trisphosphate (IP_3) and membrane-bound diacylglycerol. IP_3 triggers an early rapid increase in Ca^{2+} through its release from intracellular stores. Diacylglycerol activates protein kinase C, increasing the sensitivity of the contractile apparatus to Ca^{2+} as well as inducing intracellular signaling mechanisms that promote long-term cellular responses (proliferation and migration) through the mitogen-activated protein kinase cascade. In addition, ET activates phospholipase D and phospholipase A_2, the latter increasing the production of arachidonic acid.
and hence cyclooxygenase products (prostaglandins and thromboxanes) as well as lipoxygenase products (leukotrienes and lipoxine A₄).12

In vascular smooth muscle, ET-1 has been shown to enhance Ca²⁺ sensitivity through the activation of Rho-kinases and protein kinase C-dependent phosphorylation of the 17kDa myosin phosphatase inhibitor protein (CPI-17) pathways.13 These pathways regulate both myosin phosphorylation and dephosphorylation processes. The Ca²⁺-sensitization mechanism occurs during agonist-induced activation of the Rho-kinase or protein kinase C/CPI-17 pathway, and leads to the inhibition of the myosin light-chain phosphatase. Rho-kinase inhibits myosin light-chain phosphatase activity by phosphorylating the myosin-binding subunit (MYPT1) of the myosin light-chain phosphatase. Alternatively, CPI-17 phosphorylation also results in inhibition of the myosin light-chain phosphatase activity, which in turn maintains steady state tension in smooth muscle cells.14 In contrast, CPI-17 dephosphorylation facilitates relaxation. Activated CPI-17 protein has been shown to mediate cell proliferation by increasing and extra-cellular signal-regulated kinases (ERK) phosphorylation.15 Moreover, ET-1 has been documented to enhance vascular endothelial growth factor (VEGF) mRNA expression via activation of ET₄ receptors in vascular smooth muscle cells.16–18 VEGF levels are also increased in PH patients. This growth factor acts as a potent mitogen and chemotactarant, through its transmembrane tyrosine kinase receptor which activates major proliferative signaling pathways such as the ras-mitogen activated protein kinase cascade, resulting in proliferation, migration, and resistance to apoptosis.19

Clinical assessment of dietary supplementation of omega-3 polyunsaturated fatty acids (n-3 PUFA) including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has shown their beneficial impact in a wide range of cardiovascular diseases.20 One explanation for these beneficial effects is that n-3 PUFA competes with arachidonic acid for enzymatic conversion by cyclooxygenase, lipoxygenase, and cytochrome P450 enzymes. This competition can lead to reduced formation of vasoactive arachidonic acid metabolites while alternative PUFA-metabolites originating from DHA and EPA are increased. In addition, EPA and DHA have been reported to reduce the expression of genes for interleukins, vascular cell adhesion molecule-1, intracellular adhesion molecule-1, endothelial adhesion molecule, and E-selectin.21–23 Recently, our group has synthesized a new DHA monoacylglyceride derivative in order to assess the effect of n-3 PUFA on pulmonary arterial tone and their involvement in key components of PH pathogenesis. Fatty acids in monoacylglyceride form are generally recognized as safe and are widely used as emulsifying agents in the food industry. The aim of the present study was to evaluate the effects of DHA monoacylglyceride, namely DHA monoacylglyceride (MAG-DHA), on vasoconstriction and proliferation induced by ET-1 in human pulmonary arteries (HPA). The role and implication of CPI-17 protein was also determined in vasoconstriction and proliferation of smooth muscle cells. Herein, we report the first evidence that MAG-DHA decrease the phosphorylation level of CPI-17 leading to a reduction in both Ca²⁺-hypersensitivity and proliferation of HPA smooth muscle cells.

METHODS

Procurement of HPA. The study was approved by our institution’s ethics committee (Protocol number CRC 05-088-R2) and consent was obtained from each subject. Human lung tissues were obtained from 16 patients undergoing surgery for lung carcinoma. Isolation and culture of HPA were performed as previously described.21 Isolation and culture of HPA myocytes. HPA were dissected and placed in Hank’s balanced salt solution containing antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin). Vessels were cut longitudinally and dissected into small fragments. Tissues were transferred in a tube containing Hank’s balanced salt solution supplemented with 0.1% type IV collagenase and 0.05% type IV elastase at 37 °C during 45 min. The solution was then centrifuged and the pellet suspended in 5 ml of DMEM-F12 medium supplemented with 10% fetal bovine serum and 0.3% antibiotics. Culture flasks were placed in a 37°C incubator (5% CO₂). To validate the quality of our smooth muscle cell preparation, α-actin smooth muscle staining were performed cells and revealed that 95% of cells were positive for this marker (see Supplementary Figure S1 online).

Isometric tension measurements. The mechanical effects induced by specific agonists were measured as previously described.24,25 Paired rings of similar weight and length (inner diameter of 0.5–0.8 mm) were microdissected from the same pulmonary artery segment. Arterial ring were mounted in isolated organ baths, containing 6 ml of Krebs’ solution at 37 °C, gassed continually with the 95% O₂; 5% CO₂ mixture and to which an initial load of 0.8 g was applied. Tissues were allowed to equilibrate for 1 h in Krebs’ solution and washed out every 15 min. Passive and active tensions were assessed using transducer systems (Radnoti Glass Tech., Monrovia, CA) coupled to Polyview software (Grass-Astro-Med Inc., West Warwick, RI) for facilitating data acquisition and analysis. Induced myofilament Ca²⁺ sensitivity was assessed on β-escin–permeabilized arterial rings as previously reported.24

Western blot analysis. Western blots using specific antibodies against phospho-ERK1/2, ERK1/2, phospho-CPI-17, CPI-17, phospho-MYPT1, MYPT-1, and β-actin proteins were performed on homogenate fractions as previously described.25 Immunostaining of the blots were digitized and analyzed with Lab-Image software 2.7.

Data analysis and statistics. Results are expressed as means ± s.e.m., with n indicating the number of experiments. Statistical analyses were performed using Sigma Plot 11 and SPSS 14.0 (SPSS-Science, Chicago, IL) via one-way ANOVA followed by Dunnett’s post-hoc test. Differences were considered statistically significant when P < 0.05.24
RESULTS
MAG-DHA decreases ET-1–induced tension and Ca\(^{2+}\) sensitivity
Experiments were designed to assess the effect of MAG-DHA treatment on pulmonary arterial smooth muscle tension induced by ET-1. MAG-DHA was synthesized as previously described\(^{26}\) and the chemical structure is shown in Figure 1a. HPA explants were cultured for 24 h in the absence or presence of 5 nmol/l ET-1 plus increasing concentrations of MAG-DHA (0.1–30 µmol/l) and subsequently challenged with 5-hydroxytryptamine. The cumulative concentration–response curve to MAG-DHA (0.1–30 µmol/l) displayed a concentration-dependent inhibitory effect in ET-1–treated HPA and revealed that 3 µmol/l treatment during 24 h was sufficient to completely abolish the tension induced by 5-hydroxytryptamine (Figure 1b). MAG-DHA treatments have similar effect in the presence of 5-HT (see Supplementary Figure S5 online). To evaluate putative non-specific effects of MAG-DHA on HPA reactivity, a corn oil monoacylglyceride of similar structure was used as negative control. Results demonstrate that in the presence of 3 µmol/l corn oil monoacylglyceride, no effect was quantified on the reactivity to 5-hydroxytryptamine in ET-1–treated HPA, as compared to control tissues. These results confirm that MAG-DHA likely exhibits specific effects on HPA. Moreover, MAG-DHA has no effect on acute tension induced by 5-HT in HPA (see Supplementary Figures S2 and S4 online.) Comparative analyses were performed on β-escin–permeabilized preparations to assess the effect of MAG-DHA on Ca\(^{2+}\) sensitivity in ET-1–treated HPA. Cumulative concentration–response curve to free Ca\(^{2+}\) on permeabilized arterial rings obtained from control and ET-1–treated HPA in the absence or presence of 3 µmol/l MAG-DHA–treated HPA are shown in Figure 1c. Data analysis demonstrates that 3 µmol/l MAG-DHA pretreatment induced a shift in the half maximal effective concentration value (EC\(_{50}\) = 0.64 µmol/l) toward higher Ca\(^{2+}\) concentrations and thus reduced the Ca\(^{2+}\) hypersensitivity developed in ET-1–treated tissues (EC\(_{50}\) = 0.13 µmol/l). In contrast, there was no difference in Ca\(^{2+}\) sensitivity between tissues treated with ET-1 + 3 µmol/l MAG-DHA and control HPA, with EC\(_{50}\) values of 0.64 and 0.69 µmol/l, respectively (Figure 1c). Moreover, no difference was observed between control and 3 µmol/l MAG-DHA–treated HPA alone.

Effect of MAG-DHA treatment on ET-1–induced VEGF expression in HPA
ET-1 has been shown to enhance VEGF expression via the activation of ETA receptors in vascular smooth muscle cells.\(^{16–18}\) ET-1 is also involved in proliferation, migration, and resistance to apoptosis.\(^{19}\) To assess the effect of MAG-DHA treatment on VEGF expression levels, western blot analyses were performed on homogenates derived from HPA in controls and ET-1–treated conditions in the absence and presence of MAG-DHA, as well as in 3 µmol/l MAG-DHA–treated HPA alone. Figure 2a demonstrates that ET-1 treatment increased the density of the VEGF immunoreactive band as compared to the level detected in control (untreated) condition. However, the expression level of VEGF was reduced upon MAG-DHA pretreatment on ET-1–treated HPA. No difference was observed between control and 3 µmol/l MAG-DHA–pretreated HPA. Quantitative analysis of identical immuno-blot membrane areas were normalized as a function of β-actin staining in the corresponding fraction. As reported in Figure 2b, pretreatment of HPA explants with 3 µmol/l MAG-DHA for 24 h significantly reduced the staining density ratio of
MAG-DHA Reduces ET-1 and VEGF Effects in HPA

VEGF/β-actin, when compared to ET-1-stimulated HPA. We also demonstrate that MAG-DHA decreased the cell proliferation and Ca^{2+} sensitivity induced by VEGF treatments (see Supplementary Figure S3 online).

**Effect of VEGF inhibitor on ET-1 induced Ca^{2+} sensitivity in HPA**

Experiments were designed to determine the role of VEGF in Ca^{2+} sensitivity triggered by ET-1 in HPA using 0.3 μmol/l of VEGF Receptor Tyrosine Kinase Inhibitor II (VEGF-R inh.). The VEGF-R inh. inhibits the kinase activities of KDR, Flt-1, and c-Kit with IC_{50} values of 20, 180, and 240 nmol/l, respectively. Cumulative concentration–response curve to free Ca^{2+} concentrations on β-escin–permeabilized arterial rings obtained from ET-1–treated HPA in the absence or presence of either 0.3 μmol/l VEGF-R inh., 3 μmol/l MAG-DHA, or 3 μmol/l MAG-DHA plus 0.3 μmol/l VEGF-R inh. are shown in Figure 3a. Data analysis demonstrates that 0.3 μmol/l VEGF-R inhibitor treatment induced a shift in EC_{50} values (0.41 μmol/l) toward higher Ca^{2+} concentrations and thus reduced the Ca^{2+} hypersensitivity developed in ET-1–treated tissues (0.13 μmol/l). The difference in Ca^{2+} sensitivity between tissues treated with ET-1 + 3 μmol/l MAG-DHA in the absence or presence of 0.3 μmol/l VEGF-R inh. was not statistically significant, with EC_{50} values of 0.66 and 0.62 μmol/l, respectively (Figure 3a). In order to minimize the contribution of relaxing metabolite generated by endothelial cells, the effects

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**Figure 2** | Effect docosahexaenoic acid monoacylglyceride (MAG-DHA) treatment on vascular endothelial growth factor (VEGF) expression induced by endothelin-1 (ET-1) in human pulmonary arteries (HPA). (a) Proteins from distinct homogenates derived from control (untreated), ET-1, ET-1 + 3 μmol/l MAG-DHA and from 3 μmol/l MAG-DHA treated HPA were stained using specific antibodies against VEGF and β-actin. A decrease in staining of VEGF bands was observed in MAG-DHA treated tissues when compared to ET-1–treated HPA. Results are representative of six similar experiments. (b) Quantitative analysis of various VEGF density ratios. Staining densities in the homogenates were expressed as a function of β-actin signals (n = 6, *P < 0.05).

**Figure 3** | Effect of vascular endothelial growth factor (VEGF) inhibitor and docosahexaenoic acid monoacylglyceride (MAG-DHA) treatments on Ca^{2+} sensitivity and 17 kDa protein kinase C-potentiated inhibitor protein (CPI-17) phosphorylation level. (a) Cumulative concentration–response curve to free [Ca^{2+}] obtained from β-escin–permeabilized tissues in endothelin-1 (ET-1), ET-1 + VEGF inhibitor, ET-1 + 3 μmol/l MAG-DHA as well as in ET-1 + 3 μmol/l MAG-DHA and VEGF inhibitor–treated human pulmonary arteries (HPA). Each point represents the mean ± SEM, n = 12 for each experimental condition. *P < 0.05. (b) Representative western blot and quantitative analysis of homogenate protein fractions derived from control, ET-1, ET-1 + VEGF inhibitor, ET-1 + 3 μmol/l MAG-DHA and ET-1 + 3 μmol/l MAG-DHA + VEGF inhibitor–treated human pulmonary arteries (HPA). Each point represents the mean ± SEM, n = 6, *P < 0.05.
of indomethaen (COX inhibitor) and l-NAME (NOS inhibitor) were assessed in the presence of MAG-DHA. Note that the enzymatic inhibitors have no effect on MAG-DHA responses (see Supplementary Figure S6 online). To further investigate the putative processes potentially supporting this negative feedback mechanism induced by MAG-DHA on Ca\(^{2+}\)-tension relationship, western blot analyses were performed to assess the status of the regulatory CPI-17 protein in homogenates derived from control and ET-1–treated HPA in the absence or presence of MAG-DHA and VEGF inhibitor.

Figure 3b demonstrates that ET-1 treatment increased the density of the phosphorylated forms of CPI-17 and MYPT-1, although in the presence of 0.3 µmol/l VEGF-R inhibitor, these phosphorylated forms of CPI-17 and MYPT-1 were reduced. Moreover, the phosphorylated CPI-17 bands were reduced upon 3 µmol/l MAG-DHA treatment and no differences were observed in the presence of VEGF-R inhibitor. Total CPI-17 form staining was fairly constant from one preparation to the other (Figure 3b). As shown in Figure 3b, a 24-h pretreatment of HPA explants with 3 µmol/l MAG-DHA largely reduced the P-CPI 17/CPI-17 staining density ratio, when compared to the ratio in ET-1–pretreated HPA (bottom panel). No additional inhibitory effect was observed between MAG-DHA and MAG-DHA + VEGF-R inhibitor-treated tissues.

**Effect of MAG-DHA treatment on vascular smooth muscle cell proliferation**

To assess the role of MAG-DHA on vascular smooth muscle cell proliferation, a \(^{3}H\)Thymidine incorporation proliferation assay was performed in control (untreated) and treated HPA smooth muscle cells transfected or not with CPI-17 siRNA. Results demonstrate that ET-1 treatment enhanced cell proliferation by 48% as compared to the control condition; however, MAG-DHA or VEGF-R inhibitor treatment decreased cell proliferation induced by ET-1 (Figure 4a). To investigate the putative role of CPI-17 in ET-1–induced cell proliferation, a siRNA against CPI-17 transcripts was used as a Biochemical inhibitor. As shown in Figure 4a, CPI-17 siRNA-transfected cells displayed a marked inhibition of cell proliferation level as compared to ET-1–treated smooth muscle cells. Moreover, the combined treatment with ET-1 and MAG-DHA in CPI-17 siRNA-transfected cells revealed a cell proliferation level similar to ET-1 + MAG-DHA treated cells. Together these results indicate that MAG-DHA impairs VEGF and CPI-17 signaling, thus reducing ET-1–induced proliferation of pulmonary artery smooth muscle cells.

The phosphorylated active form of CPI-17 has recently been shown to enhance ERK phosphorylation levels and increase cell proliferation.\(^{15}\) Western blot analyses were therefore performed to determine the status of CPI-17, MYPT-1 and ERK in smooth muscle cells upon ET-1 and MAG-DHA treatment. Data revealed that ET-1 treatment increased the phosphorylation levels of CPI-17, MYPT-1, and ERK when compared to the phosphorylation level detected in control (untreated) cells (Figure 4b). However, 3 µmol/l MAG-DHA treatment resulted in reduced phosphorylation levels of CPI-17, MYPT-1 and ERK in ET-1–treated smooth muscle cells (Figure 4b). In the presence of MAG-DHA and VEGF inhibitor, no additional reduction of CPI-17, MYPT-1 and ERK phosphorylation levels was detected in ET-1–treated cells. Figure 4c illustrates the proposed mode of action of MAG-DHA on cellular mechanisms involved in Ca\(^{2+}\) sensitivity and proliferation induced by ET-1 in HPA. According to our experimental data, following ET-1 treatment, the addition of MAG-DHA reduces VEGF expression levels which results in decreased CPI-17 phosphorylation levels leading to diminished Ca\(^{2+}\) sensitivity. On the other hand, MAG-DHA, through its interaction with VEGF signaling, also inactivates CPI-17 which results in a reduction in ERK phosphorylation levels and a concomitant decrease in smooth muscle cell proliferation.

**DISCUSSION**

**MAG-DHA prevents ET-1 induced tension and Ca\(^{2+}\)-sensitivity in HPA**

ET-1 is a potent mitogenic and vasoconstrictor peptide that mediates pulmonary vascular tone.\(^{3}\) The lung is considered as the major site for ET-1 production and clearance.\(^{27,28}\) In all established animal models of pulmonary hypertension, ET-1 levels are elevated in circulating plasma.\(^{3}\) Moreover, several studies have reported that ET-1 is implicated in human PH, since expression of ET-1 is increased in the lungs of patients with pulmonary hypertension\(^{29}\) and plasma levels of ET-1 are also elevated in patients with idiopathic pulmonary hypertension.\(^{29}\) Nevertheless therapeutic options for pulmonary hypertension remain limited despite the introduction of prostacyclin analogs, endothelin receptor antagonists and phosphodiesterase 5 inhibitors over the past 15 years. Indeed, these interventions predominantly address the endothelial and vascular dysfunction associated with the condition, but merely delay the progression of the disease rather than offer a cure.\(^{30}\) In this study, we evaluated the ability of MAG-DHA, a DHA monacylglyceride derivative compound, to reduce the vasoconstriction induced by ET-1 in HPA. Fatty acids in monacylglyceride form are generally recognized as safe and are widely used as emulsifying agent in the food industry. This monacylglyceride of DHA was demonstrated to increase the oral bioavailability of DHA compared to commercially available marine oil.\(^{26,31}\) Our data revealed that low concentration of MAG-DHA decreased the over-reactivity triggered by 5-hydroxytryptamine following ET-1 pretreatment in human distal pulmonary arteries. A similar strategy has been already validated in human bronchi following tumor necrosis factor-α treatment and methacholine challenges.\(^{31}\) Several studies have demonstrated that Ca\(^{2+}\) sensitizing mechanisms may also be primed under pathophysiological conditions by various mediators, such as ET-1, eicosanoids, and that this Ca\(^{2+}\) sensitization processes is involved in pulmonary hypertension.\(^{32–34}\) Hence, it was therefore of potential clinical interest to find a specific agent that could significantly oppose the leftward shift in Ca\(^{2+}\) sensitivity induced by ET-1. Our data demonstrate that a 24 h pretreatment with MAG-DHA was able to reduce the Ca\(^{2+}\) hypersensitivity developed by ET-1–pretreated HPA.
Moreover, our findings establish that MAG-DHA interacts with CPI-17 pathway in order to decrease Ca$^{2+}$ sensitivity of arterial smooth muscle.

Implication of VEGF in Ca$^{2+}$ sensitivity and proliferation

Advanced PH is characterized by extensive vascular remodeling that is usually resistant to vasodilator therapy. As the major component of the vascular media, pulmonary arterial smooth muscle cells are the main effectors of the physiological responses during pulmonary vascular remodeling. VEGF is an endothelial cell-specific mitogen and a potent angiogenic peptide, which is secreted by a variety of cell types. VEGF has been shown to be involved in several physiological and pathological processes that require proliferation of endothelial and smooth muscle cells. It has been reported that VEGF plays an important role in the development of pulmonary hypertension. Serum VEGF concentrations are elevated in patients with PH and are likely related to an increase in VEGF production at sites of vascular injury due to tissue hypoxia. In turn, these elevated levels enhance dysregulated angiogenesis in the pulmonary vasculature, leading to the development of PH.

Omega-3 fatty acids have been demonstrated to alter the transcription of specific genes involved in lipogenesis, glycolysis, synthesis of glucose transporters, inflammatory mediators, early response genes and genes for cell adhesion molecules. EPA and DHA reduce the expression of genes for interleukin (interleukin-6, interleukin-8, interleukin-1β) vascular cell adhesion molecule-1, intracellular adhesion molecule-1, endothelial adhesion molecule, and E-selectin. In the present study, we

Figure 4 | Effect of docosahexaenoic acid monoacylglyceride (MAG-DHA) on vascular endothelial growth factor (VEGF)-induced proliferation of pulmonary arterial smooth muscle cells (PASMC). (a) Bar graph displaying mean $[^{3}H]$thymidine incorporation in control, 17 kDa protein kinase C-potentiated inhibitor protein small interfering RNA (CPI-17 siRNA) transfected cells treated or not with 5 nmol/l endothelin-1 (ET-1). $[^{3}H]$Thymidine incorporations were also determined in the presence of 3 µmol/l MAG-DHA and 0.3 µmol/l VEGF receptor inhibitor. (b) Western blot analysis of phosphorylated forms of CPI-17 (P-CPI-17), MYPT-1 (P-MYPT-1), ERK (P-ERK1/2) as well as total CPI-17, myosin-binding subunit of the myosin light chain phosphatase (MYPT-1) and extracellular signal-regulated kinases (ERK) protein detection. Results are representative of six independent experiments. (c) Functional diagram summarizing the mode of action of MAG-DHA on intracellular mechanisms leading to Ca$^{2+}$ sensitivity and proliferation induced by endothelin-1 in human pulmonary arteries.
show that MAG-DHA which is well absorbed by cells, decreases ET-1–induced VEGF expression, leading to a reduction in Ca2+ and airway smooth muscle proliferation through an inhibition of CPI-17 protein. Moreover, DHA, EPA or their metabolites are also known to mediate vasodilatation.\textsuperscript{39,40} In a previous study, we demonstrated that 19,20-EpDPE, a cytochrome P450 epoxygenase metabolite derived from MAG-DHA, induced a concentration-dependent relaxation of smooth muscle from distal HPA and that this action was related to an inhibition of Rho-kinase pathway.\textsuperscript{24}

To our knowledge, this is the first study demonstrating that VEGF interacts with CPI-17 regulatory protein resulting in an increase in Ca2+ sensitivity and proliferation of smooth muscle upon ET-1 stimulation of pulmonary arteries. Moreover, for the first time, we demonstrate that the MAG-DHA compound is able to decrease the activation of CPI-17 leading to a reduced phosphorylation level of MYPT-1 and ERK. The ability of this compound to reduce both the Ca2+ sensitivity and proliferation of smooth muscle could therefore be of pathophysiological significance in the treatment of pulmonary hypertension. Hence, MAG-DHA may represent an emerging approach targeting the vasoconstriction and pro-proliferative phenotype that underlies pulmonary vascular remodeling in the lung. However, we cannot rule out that MAG-DHA could be metabolized in glycerol and DHA (via PLA1) and that the long chain fatty acid may be modified into epoxy or poly-hydroxyl compounds as previously reported.\textsuperscript{24,31} The present findings also warrant further studies to investigate the potential application of this DHA monooacylglyceride as an emergent compound of medicinal interest in the prevention and management of hypertension.

Supplementary material is linked to the online version of the paper at http://www.nature.com/ajh

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Disclosure: Only S.F. declares a potential conflict of interest; he is the owner of SFC Pharma, including patents.


